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CONTRACTILE PROTEIN SYSTEM OF HEART AND MUSCLE*†

During the previous century, as investigators became concerned with the structural basis of vital phenomena, two important generalizations emerged: one was that physiologically active structures always consist, in large part, of protein; the other, that fibrous structures seem to be correlated with the specialization of parts of cells for the purpose of motion. Such fiber structures are obvious in muscle cells and contractility is ascribed to the imbedded fibrils having a submicroscopic, longitudinally arranged filamentous structure.

In striated muscle cells, this is complicated further by a cross-striated differentiation. It became apparent that contractility is the function of arrays of fibrous protein molecules. These molecules have the property of responding to the cell's state of excitation and of interacting with its metabolism in such a manner that at set times they undergo topochemical reactions, utilizing the energy of metabolism for the elaboration of force. This statement includes a great deal more than can be discussed in the available space.

Within each striated muscle cell, we distinguish three main organizational entities: the mitochondrial, the sarcoplasmic, and the fibrillar systems. The mitochrondria carry all those enzymes which deal with the oxidation of metabolites and the associated phosphorylation reactions leading to the synthesis of high-energy phosphate compounds, such as adenosine triphosphate (ATP). The sarcoplasm consists of a great many soluble enzymes occupied with various metabolic processes, notably with glycolysis, by which glucose or glycogen are converted to lactate. This too is linked with the synthesis of ATP from adenosine diphosphate (ADP) and phosphate, but the yield per molecule of metabolite is far less than with oxidation. Those muscles maintaining fairly intense activity for long periods, or even indefinitely, can do so only when their oxidative ATP synthesis keeps up with the demand for this substance. Hence, we find in the most typical example of such muscle, the myocardium, rich occurrence of mitochondria and all their oxidative and phosphorylative enzymes. Whatever their relative preponderance, we find that these two metabolic systems serve the generation of high-energy phosphate.

The third system, the fibrillar structures, may be said to consume ATP, and it is the one to be discussed here with special regard to its protein constituents. Among these, myosin has received most attention. Two separate types of evidence led to the belief that myosin is really the heart of the matter: von Muralt and Edsall (1930) found streaming solutions of myosin to be birefringent (indicative of the presence of filamentous molecules), and Weber (1935) prepared artificial myosin threads showing a similar birefringence as muscle cells. Then Engelhardt and Ljubimowa (1939) found that myosin is an enzyme, ATPase, that catalyzes the hydrolysis of ATP, making it appear as a fibrous structure molecule with the ability to catalyze what was supposed to be the energy-yielding reaction — so to speak, a "contractile enzyme." This was a momentous perspective. Yet, there was no convincing evidence that ATP could cause myosin structures to contract or relax. The observations contributing to this final discovery and starting a new era of molecular physiology were made by A. Szent-Györgyi in the early 1940's.

Highly purified preparations of contractile protein can be obtained by appropriate chemical methods. Yet even these, like crude extracts, are found to be highly variable in their behavior.

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† While this article is more technical than those usually published in *Modern Concepts of Cardiovascular Disease*, readers will find it rewarding because of the fine vista it gives of how progress is being made in one of the many fascinating forefronts of Medicine. Ed.

Apart from other factors influencing the results, it was found that with certain standard technics, brief extraction yielded a form named myosin A, which in solution had relatively low viscosity, turbidity and flow birefringence; while prolonged extraction, lasting up to 24 hours, gave a myosin B, solutions of which were exceedingly viscous, turbid and birefringent, indicative of highly elongated and aggregated particles. Myosin A is not a well-defined product, as it contains varying amounts of B. Later on, Szent-Györgyi succeeded in preparing an entirely pure myosin, free of all myosin B, in crystalline form by rigorous purification of myosin A. In other words, incubation of already extracted myosin, with the muscle residue, leads to its transformation into myosin B. Straub, in 1942 and 1943, identified a new fibrous protein, actin, which combines with myosin to form myosin B, now renamed actomyosin:

$$myosin + actin \longrightarrow actomyosin. \qquad (1)$$

Both myosin and actomyosin have ATPase activity. While myosin itself is otherwise rather inert to ATP, actomyosin undergoes profound physical changes when interacting with its substrate. In solution, these changes reveal themselves by a great reduction of the viscosity or turbidity, persisting as long as any ATP remains present. This change is ascribed to the fact that ATP, when combined with (acto) myosin in the sense of an enzyme-substrate complex, reverses reaction (1) and dissociates the actomyosin complex into its constituents. This was a disappointing conclusion. It would seem that, had one found instead that ATP makes dissolved actomyosin particles contract, this would have had a direct bearing upon the physiologic problem, whereas the dissociation of the actomyosin complex would not seem to explain contractility at all. However, apart from the fact that nature's ways are unfathomable, two circumstances must be kept in mind: first, that in vivo the salt concentration (concentrated salt solutions are required to extract the contractile proteins) is so much lower that myosin and actomyosin are insoluble, and so may be unable to dissociate; and second, that with the present views regarding sliding mechanisms (see below), dissociation of the complex may not be so paradoxical at all.

If the reactions in actomyosin solutions give no direct clue as to contractility, more direct evidence was obtained by Szent-Györgyi from the behavior of actomyosin threads. These, unlike myosin threads which are quite inert, show strong contraction when exposed to ATP. This is one of the most striking biologic discoveries ever made. It must be admitted that the first experimental model had certain imperfections: the threads upon contraction became shorter and thinner, instead of thicker, and when loads were attached to them they would be plastically stretched. These imperfections could be ascribed to the deficient molecular bonding and orientation within the threads. Gradually it was learned how to improve this. Later, Szent-Györgyi (1949) developed his glycerol-extracted fiber preparation, in which the original molecular configuration was more nearly maintained; Hayashi (1952) prepared strong threads from surface-spread actomyosin, and Weber and Portzehl (1952) oriented and strengthened actomyosin threads by controlled drying and stretching. These preparations thicken in the act of shortening and can perform work; they became the subject of a series of classical investigations from Weber's laboratory in which a refined analysis was given of the connection between shortening and the splitting of ATP, to which we shall return below.

Studies on the ATPase activity of myosin and actomyosin have been exceedingly numerous, but relatively unrewarding. This is the case, partly, because from this splitting reaction between ATP with H_2O as acceptor, not much is learned of intermediate stages, and it may well be that essential in vivo stages do not even occur in the experiments with isolated enzymes. More prohibitive, however, is the enormous complexity of the problem, in terms of the sensitivity of the enzyme and the fantastic variability of its dependence upon pH, Na, K, Ca and Mg ions. These problems have been studied extensively in several laboratories, but what emerges is mainly an ever increasing complexity and an awareness of the limitations of purely kinetic studies. However, two of our recent findings may be mentioned, both dealing with the enzymatically active sites on the myosin molecule. In the first (with Hanson), an attempt was made to measure the interaction between actomyosin and ATP by determining the turbidity response caused by small amounts of ATP (less than one molecule per myosin molecule) kept intact against the ATPase action by a rephosphorylating enzyme and a reservoir substrate. In the second (with Nanninga), using pure myosin again with a rephosphorylating enzyme, the free ATP was continuously monitored by recording the light emission caused by simultaneously having firefly luciferin and luciferase in the system. Both approaches confirm each other in showing that one molecule of myosin binds one molecule of ATP with considerable affinity:

$$ATP + myosin \rightleftarrows myosin - ATP \rightarrow myosin + ADP + P. \qquad (2)$$

Such observations provide a basis for the concept, originated by our laboratory in 1948, which maintained that the binding of ATP to myosin, rather than its subsequent splitting, is the first chemical event in the contraction cycle; this later became a basic assumption in the elaborate theoretical studies of Morales. Opposed to this is

Weber's view, resulting from his studies on the contraction of fiber models, that the act of binding merely plasticizes the system, but that a supply of energy provided by splitting is needed for contraction.

Since the actin-myosin complex is so strikingly capable of showing almost vital activities, it is worth learning more about the chemical-physical properties of its constituents, through studies on myosin and actin in the pure state.

Actin was prepared by Straub from muscle subjected to several extractions. He obtained a crude solution which, upon addition of any neutral salt, became exceedingly viscous and birefringent. These changes he ascribed to the occurrence of actin in two forms: globular (G) actin in the salt-free extract, polymerizing to fibrous (F) actin by the addition of salt. This specific change became the basis of a selective way for purification of actin by differential ultracentrifugation, developed in our laboratory in 1953. Physical studies on the pure protein confirmed that the G-F transformation is a polymerization process caused by salt, because this screens the negative molecular charges preventing the molecules from colliding. The pure protein also made it possible to investigate the clues obtained in Straub's and Laki's laboratories that ATP is necessary for the polymerization reaction and is broken down during the course of it. The following stoichiometric reaction was recognized:

$$Actin + ATP \rightarrow (Actin)_{polym.} + ADP + P. \quad (3)$$

The stoichiometry of the reaction, and the amount of actin in muscle, are such that a one-time occurrence of the reaction could approximately account for the energy mobilized in a muscle twitch. The same order-of-magnitude statement would also hold for the one-time occurrence of reaction (2). Hence, reactions such as these could well be the direct basis of physiologic events, but their actual occurrence in single acts of contraction has not been demonstrated.

G-actin is a protein with a molecular weight of about 58,000 and its polymerization leads to an end-to-end aggregation process whereby single molecules, with dimensions of the order of 50 Angström units, would form linear polymers consisting of threads of about this same diameter. Filaments of such a dimension run along a large part of the sarcomere, from the Z-membrane through the I-band into the A-band, terminating at the limit of the H-zone in the A-band's center. Furthermore, Hanson and Huxley suggested that just these filaments are removed under conditions leading to the selective extraction of actin after previous removal of myosin. These filaments are placed in a regular hexagonal array.

Myosin is likewise available in pure form and can be crystallized. In view of its bulk occurrence in birefringent muscle, the question of its molecular length attracted much interest. This was determined as 1,600 Angström units from light-scattering dissymmetry (Mommaerts, 1950), the second protein to be so investigated. (The first had been tobacco mosaic virus, the length of which could be directly checked by electronmicroscopy.) Myosin's molecular weight was recently set at 420,000, based upon the approach to sedimentation-diffusion equilibrium in the ultracentrifuge studied by interferometry (Mommaerts and Aldrich, 1958). If, as seems likely, the myosin molecule is a rod, it would be 1,600 Angström units long and about 1/50 as thick.

Myosin, too, occurs in the sarcomere in a strictly localized and regularly arranged fashion, in the form of rods of about 100 Angström units thick and running the full length of the A-band. Clearly, these rods are not single myosin molecules but agglomerates of them, which might be considered as crystallites. In the parts of the A-band outside the H-zone, they interdigitate with the actin as shown in the electronmicroscopy studies by Huxley in 1957, and by Hanson and Huxley in 1955. According to the most recent studies, the regularly spaced bridges stick out from the myosin rods toward the actin rods. There may be approximately one such bridge per myosin molecule.

The sarcomere is thus constructed of an interdigitated system of parallel rods, and the current Hanson-Huxley theory of contractility enunciates that contraction is due to a sliding of the two systems of rods into each other's territories, without a change in length in either of them (at least, initially; extreme shortening may cause a piling up of filament material in the center of the A-band, causing "contraction bands"). This theory will undoubtedly be subjected to critical reinvestigation in the near future, but for the moment it is certainly the best picture we have.*

While the events in muscle have been represented as taking place between only two proteins (how refreshing in comparison with blood coagulation), there may be more. Apart from special proteins described for invertebrate smooth muscle, there is in muscle and heart a protein, tropomyosin, discovered by Bailey. Its physical properties are fully as active as those of actin in the reverse: it polymerizes in the absence of salt, but so far no interactions of it with ATP or with other muscle proteins have been described. There is also Amberson's delta-protein in muscle, which can compete with actin for myosin. We have no information concerning the role and location of these proteins.

Myosin itself, as discovered by Gergely, Mihaly and A. G. Szent-Györgyi, can be split by

* While this section has been written from the viewpoint of the Hanson-Huxley theory, it must be mentioned that current work by Sjöstrand may reopen the question of its applicability to living muscle.

proteolytic enzymes into well-defined products, meromyosin -H and -L. The former retains the affinity toward actin, ATP and the ATPase activity; the latter resembles myosin in its solubility but has no affinity to actin and ATP. Neither one resembles tropomyosin. The molecular dimensions of these products have not been accurately determined, but it is likely that the myosin molecule contains one of each. More drastic means lead to further splitting. Many proteins can be decomposed to nonequivalent fragments, and these findings do not yet deny that myosin is the physiologic entity. However, there may be ample reason to reinvestigate the nature of myosin and to scrutinize more closely the question of its actual occurrence in vivo and of any possible changes it may undergo during physiologic activity.

So far, all knowledge of the muscle proteins have been derived from studies on skeletal muscle, mostly from rabbits. Studies on myocardial proteins have been far less numerous, although there is occasional mention of myosin, actin or tropomyosin having been prepared from mammalian hearts, and of cardiac myosin having a considerably lower ATPase activity. On the whole, the literature on the cardiac proteins allows little more than the conclusion that myosin and actin occur here, that actomyosin can be prepared with similar responses to those obtained from skeletal muscle, and that glycerol extracted cardiac fibers can contract with ATP. These facts, and their interesting connections with cardiac physiology and pathology, are presented in the publications of Benson et al. (1955), and Taeschler and Bing (1953).

Recently, Ellenbogen and Olson have raised the controversial question as to whether the heart in vivo contains any myosin (as commonly understood) at all, but inasmuch as the evidence is not yet published in full detail, we must suspend judgment. It is clear, however, that a definite characterization of the cardiac proteins must first be achieved before specific cardiologic problems can be initiated.

It is not possible to discuss here the mechanisms of contractility, the contractile activity of extracted fiber preparations and their reversal by relaxation factors, nor to analyze the relation between the properties of the actomyosin and the biochemical and mechanical events occurring in vivo. However, even this limited assay may give an impression of the great advances in this field. It is quite appropriate to end with a word of recognition of the contributions made by the pioneer worker, Albert Szent-Györgyi, whose experimental savoir-faire changed perspectives and wish-dreams into tangible facts.

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